Technical Abstract

A phase I trial of B7-transfected allogeneic melanoma cell lines to induce cell-mediated immunity against tumor-associated antigens presented by HLA-A2 or HLA-A1 in patients with stage IV melanoma.

BRMP 9401
NCI Protocol T93-0161

Biological Response Modifiers Program, DCT, NCI

The scientific basis for this protocol is derived from two main points. Recent data indicate that two stimuli are required to activate T cells to proliferate and secrete cytokines. Signal one is provided by the T cell receptor, and the other requires interaction of other T cell surface receptors with their ligants on antigen presenting cells. The second point, is that in the case of human melanoma, tumors from many patients express the same tumor antigens, and that peptides derived from these tumor antigens are often presented on tumor cell surfaces by MHC class I molecules HLA-AI or A2. Thus, it may be possible to induce immunity against these shared melanoma antigens by vaccination with allogeneic tumor cell lines that express the tumor antigens and are HLA-A1 or HLA-A2 positive. In order to study these questions in a clinical trial of advanced stage melanoma patients, we introduced the gene encoding the T cell co-stimulatory molecule B7 (CD28 ligand) into 3 human melanoma cell lines. These tumor lines are HLA-A2+ (one is HLA-A1+), express significant levels of LFA-3 and ICAM-I (and in one case HLA-DR) on their surfaces, and produce RNAs encoding the shared melanoma antigens MAGE-I and -3, and tyrosinase. We have examined the stimulatory capacity of parental tumor lines and B7-transfectants (DM150/B7-8, DM13/B7-7, and DM93/B7-4) in 7 day primary mixed lymphocyte cultures (MLC) with allogeneic human T cells obtained from PBL of normal donors or melanoma patients. In multiple experiments, parental tumor lines (which do not express detectable B7) fail to induce significant activation of allogeneic T cells as determined by lack of increased expression of HLA-DR or CD25 on T cell surfaces. In contrast, B7-transfected lines induce increased expression of HLA-DR and CD25 (in both CD4+ and CD8+ subsets), and a 5-10 fold increase in T cell number compared to cultures with parental cell lines. CTL induction in primary MLC was determined in this system by 7d co-culture of allogeneic T cells with the parental DM150 or DM150-B7 cell lines. While T cells cultured with DM150 exhibit only background levels of lytic activity, T cells cultured with DM150-B7 lyse this line, and also the unmodified parental line and the HLA-A2+ DM13 cell line. TIL lines that recognize the shared melanoma antigen(s) presented by HLA-A2 lyse all three parental lines, providing functional data that these lines present shared melanoma antigens via the endogenous MHC class I pathway. These data indicate: 1. B7 is expressed by all three lines and is biologically functional as assessed by the ability to activate resting human T cells. 2. The cell lines express the genes encoding the known shared melanoma antigens and are lysed by HLA-A2 specific TIL derived from melanoma patients, demonstrating functional expression of the shared melanoma antigens by these lines. These studies form the preclinical basis for a vaccine trial in which patients will be vaccinated with lethally irradiated allogeneic melanoma cell lines genetically engineered to express human B7. The three lines will be injected subcutaneously at two week intervals for three vaccinations, followed by 3 injections at monthly intervals. The cell lines will be given on a rotating basis. Cohorts of patients will receive escalating doses of 10^7, 10^8, or 10^9 cells. This is a Phase I trial to determine the MTD of this therapy, but immunologic parameters such as generation of CTL precursors in peripheral blood and draining lymph nodes will also be monitored.